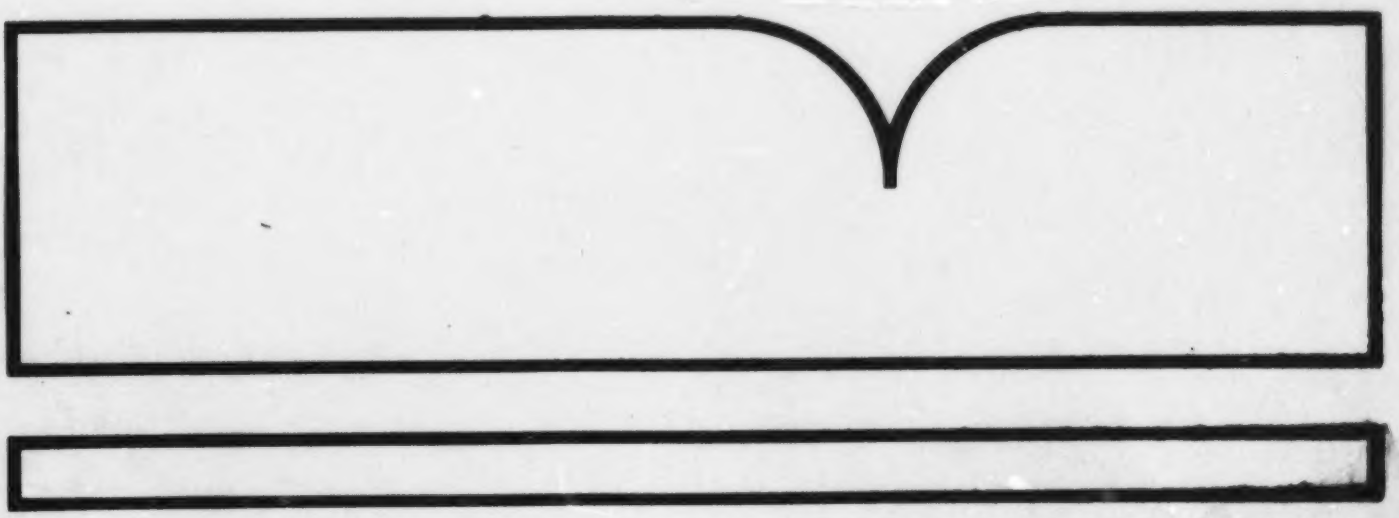
PB82-233644

Pulmonary Injury in Rats Following Continuous Exposure to 60 Percent 02 for 7 Days

(U.S.) Health Effects Research Lab.
Research Triangle Park, NC

5 Jun 81



U.S. Department of Commerce
National Technical Information Service
NTIS

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA-600/J-81-032	2. JOURNAL ARTICLE	3. RE PB82-233644
4. TITLE AND SUBTITLE: Pulmonary injury in rats following continuous exposure to 60% O_2 for 7 days		5. REPORT DATE 6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S): G. Hayatdavoudi, J.J. O'Neil, B.E. Barry, B.A. Freeman, and J.D. Crapo		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS: Department of Medicine, Duke University, Durham 27710; and Environmental Protection Agency, Research Triangle Park, North Carolina 27711		10. PROGRAM ELEMENT NO. A9GA1A 11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS: Office of Research and Development, Health Effects Research Laboratory, US Environmental Protection Agency, Research Triangle Park, NC 27711		13. TYPE OF REPORT AND PERIOD COVERED 14. SPONSORING AGENCY CODE EPA-600/11

15. SUPPLEMENTARY NOTES

Published in: J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 51(5): 1220-1231, 1981

16. ABSTRACT

Morphological, biochemical, and physiological studies were done on rats exposed to 60% O_2 for 7 days. This exposure did not induce O_2 tolerance but instead caused a significant decrease in survival time of animals subsequently exposed to pure O_2. The activity of lung superoxide dismutases and glucose-6-phosphate dehydrogenase were unchanged after exposure to 60% O_2. A decrease in lung compliance was suggested by changes in the total lung capacity and in the pressure-volume curves of excised lungs. Ventilation of these animals with large tidal excursion resulted in pulmonary edema. Morphometric analyses revealed a significant decrease in alveolar air volume and an increase in the number of alveolar macrophages. The most significant lesions involved the pulmonary vascular bed. The volume and thickness of the capillary endothelium was decreased. There were focal areas of pericapillary fluid accumulations, and a number of the smaller vessels had perivascular edema. These findings suggest that significant pulmonary injury occurs in rats exposed to 60% O_2 and that the primary site of injury is the pulmonary capillary endothelium.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA 22161

18. DISTRIBUTION STATEMENT: Release to Public	19. SECURITY CLASS *(This Report)* Unclassified	21. NO. OF PAGES
	20. SECURITY CLASS *(This page)* Unclassified	22. PRICE

EPA-600/J-81-032
JOURNAL ARTICLE

Pulmonary injury in rats following continuous exposure to 60% O_2 for 7 days

GHOLAMHOSSAIN HAYATDAVOUDI, JOHN J. O'NEIL, BRENDA E. BARRY, BRUCE A. FREEMAN, AND JAMES D. CRAPO
Department of Medicine, Duke University, Durham 27710; and
Environmental Protection Agency, Research Triangle Park, North Carolina 27711

HAYATDAVOUDI, GHOLAMHOSSAIN, JOHN J. O'NEIL, BRENDA E. BARRY, BRUCE A. FREEMAN, AND JAMES D. CRAPO. *Pulmonary injury in rats following continuous exposure to 60% O_2 for 7 days.* J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 51(5): 1220–1231, 1981.—Morphological, biochemical, and physiological studies were done on rats exposed to 60% O_2 for 7 days. This exposure did not induce O_2 tolerance but instead caused a significant decrease in survival time of animals subsequently exposed to pure O_2. The activity of lung superoxide dismutases and glucose-6-phosphate dehydrogenase were unchanged after exposure to 60% O_2. A decrease in lung compliance was suggested by changes in the total lung capacity and in the pressure-volume curves of excised lungs. Ventilation of these animals with large tidal excursion resulted in pulmonary edema. Morphometric analyses revealed a significant decrease in alveolar air volume and an increase in the number of alveolar macrophages. The most significant lesions involved the pulmonary vascular bed. The volume and thickness of the capillary endothelium was decreased. There were focal areas of pericapillary fluid accumulations, and a number of the smaller vessels had perivascular edema. These findings suggest that significant pulmonary injury occurs in rats exposed to 60% O_2 and that the primary site of injury is the pulmonary capillary endothelium.

oxygen adaptation; superoxide dismutase; morphometry; pulmonary edema; pulmonary function

SHORTLY AFTER his discovery of O_2 in 1775, Priestly postulated the possible hazards of the inhalation of high concentrations of this gas (25). In 1783, Lavoisier discovered the inflammatory effects of O_2 and described congestion and consolidation of the lungs of guinea pigs that inhaled high concentrations of O_2 (19). The description of the pathological findings of pulmonary O_2 toxicity is usually credited to J. Lorraine Smith who, in 1899, reported studies of pulmonary O_2 toxicity in normobaric and hyperbaric environments (31). These and subsequent studies clearly demonstrate the toxicity of O_2 to mammalian lungs, but the lowest dose and duration of O_2 exposure that will cause significant pulmonary injury is not clearly established.

A number of investigators have found O_2 concentrations of 60% and below to cause no major alteration in pulmonary structure and function (1, 2, 17). Others (18, 20) have claimed that exposures to concentrations below 40% could harm the respiratory system. The toxic effects of lower concentrations of O_2 are also interrelated with the process of O_2 adaptation. In several animal models, it has been shown that exposure to sublethal doses of O_2 leads to the development of O_2 tolerance (3, 11). When adult rats are exposed to 100% O_2, they die within 60–72 h of exposure. If rats are first exposed to 85% O_2, they survive and, after 5–7 days of exposure, can be transferred to 100% O_2, which they will then tolerate for prolonged periods of time. Although these animals are tolerant to the lethal effects of pure O_2, their lungs are extensively damaged, and they are cyanotic, lethargic, anorectic, and have lost a significant portion of their body weight (10, 11). It is not known whether the process of O_2 adaptation in rats can be stimulated by concentrations of O_2 below 85%. In addition, the threshhold of O_2 exposure in rodents below which no significant lung injury will occur has not been established. To study these questions we carried out the following studies on the lungs of rats exposed to 60% O_2 for 7 days.

METHODS

Animals. The animals used were 10 wk old, specific pathogen-free male Charles River CD-strain rats, weighing 300–350 g. All control and treated groups of animals were weight matched at the beginning of the experiments. They were maintained in 12-h-on, 12-h-off light cycles and were fed Wayne Sterilizable Lab-Blox ad libitum.

Exposures. All O_2 exposures were continuous and were carried out in polystyrene chambers at 23–25°C according to methods previously described (11). The various O_2 concentrations were achieved by mixing air and pure O_2 and providing a sufficient gas flow to achieve six to eight complete gas changes per hour. Humidity was maintained between 60–80%. The O_2 concentration was continuously monitored in the cages and varied less than 2%. The CO_2 concentration remained below 0.5%. Control animals were kept in air under identical exposure conditions.

Tissue preparation for biochemical studies. For enzyme assays, the animals were anesthetized with intraperitoneal pentobarbital sodium 50 mg/kg body wt. The chest was then opened, the inferior vena cava and the abdominal aorta were transected, and after 30–60 s the lungs were excised.

The supernatant fraction of a whole-lung homogenate was prepared by dicing both lungs from each rat and

then homogenizing them in 3 vol of cold 0.01 M K_2HPO_4 buffer with 10^{-3} M ethylenediaminetetraacetic acid (EDTA) at pH 7.8 using six strokes of a Potter-Elvehjem-type glass and Teflon homogenizer. This homogenate was sonicated at a power of 70 W for 45 s and then further diluted. The supernatant fraction was separated by centrifugation at 20,000 *g* for 10 min at 4°C. For superoxide dismutase (SOD) assays, the above homogenization was done in the presence of 2% Triton X.

Chemical analyses. SOD was assayed by the cytochrome *c* assay at both pH 10.0 and pH 7.8 (7, 8). In crude homogenates of mammalian tissues, two different dismutases are known to be present. The rat copper-zinc SOD, found predominantly in the cytosol, has been isolated and is 9 to 10 times more active in the pH 10.0 assay than it is in the pH 7.8 assay (7). The manganese SOD, which is predominantly in the mitochondria (14), has also been isolated from rat tissues; it differs from the copper-zinc enzyme in that it has no increase in activity in the pH 10.0 assay relative to the pH 7.8 assay (27). The distribution of the two enzymes is not known to be absolute, and it is difficult to distinguish the manganese and the copper-zinc dismutases in crude homogenates. Unless it is present in unusually large amounts, the manganese enzyme contributes relatively little to the total activity seen in the pH 10.0 assay. Thus this assay is useful to estimate changes in the copper-zinc SOD (8). To evaluate the manganese SOD the pH 7.8 assay must also be used. The use of this assay for crude homogenates is complicated by the following factors. *1*) Both of the dimutases are highly active. *2*) Cytochrome oxidase gives an apparent SOD activity (8). *3*) There are nonspecific inhibitors of the assay in crude homogenates. Cytochrome oxidase is not active at pH 10.0 and therefore does not interfere with that assay. The problem of cytochrome oxidase interference in the pH 7.8 assay has been overcome by taking advantage of its extreme sensitivity to cyanide (8). Cytochrome oxidase is 100% inhibited by 10^{-5} M KCN, whereas neither of the rat dismutases are affected by this level of KCN. Boiling for 20 min totally inactivated both the copper-zinc and the manganese dismutases. Boiled, crude rat lung homogenates still cause a decrease in the uninhibited rate of reduction of cytochrome *c* that is independent of the SOD effect. Appropriate corrections for this effect were made when calculating the SOD activity in crude homogenates.

Glucose-6-phosphate dehydrogenase (G-6-PD) was assayed according to the method of Lohr and Waller (21). Protein was assayed according to the method of Lowry et al. (22).

Techniques for the determination of O_2 consumption ($\dot{Q}O_2$) and substrate oxidation by 1.0-mm rat lung tissue slices have been described in detail (23). These slices were prepared from animals that had been briefly anesthetized with < 5% halothane. $\dot{Q}O_2$ was stimulated in some of these studies by the addition of 37 mg/l of 2-4-dinitrophenol to the incubation medium to uncouple mitochondrial oxidative phosphorylation.

Tissue preparation for histological studies. After the exposures, each rat was anesthetized with pentobarbital sodium ip, and its trachea was cannulated. Then, in rapid succession, the abdomen was opened, both diaphragms were punctured to allow collapse of the lungs, and 2% glutaraldehyde in cacodylate buffer at pH 7.4 and with a total osmolality of 350 (34) was infused into the trachea. The fixative was maintained at a constant pressure of 20 cm above the midpoint of the chest wall. The lungs were allowed to fix for 30 min in situ with a closed chest wall, after which they were carefully dissected, and the total lung volume was determined both by volume displacement and by weighing the lungs suspended in a beaker of glutaraldehyde fixative (28).

Light microscopy. Slices 2-mm thick were cut from the left lung of each rat and embedded in paraffin, and 7-μm sections were prepared. In addition, the right middle lobe from each rat lung was embedded in paraffin, and 10-μm sections were step cut at 1.5-mm intervals. All of these sections were stained with hematoxylin and eosin following standard procedures for light microscopic sections. In addition, the left lung of each of the animals reported in Table 8 was fixed in Formalin without intratracheal inflation, and paraffin sections were prepared and stained with hematoxylin and eosin.

Electron microscopy. Two-millimeter cubes were taken from four sites in the left lung and were kept in 2% glutaraldehyde fixative for 4 h before being washed in cacodylate buffer. The sites sampled were upper anterior, upper posterior, lower anterior, and lower posterior. In each case, the tissue samples were taken near the pleural margin of the lung but did not include pleura.

After the specimens were washed in cacodylate buffer, they were postfixed with 2% OsO_4 for 90 min, rinsed again in buffer, and subsequently dehydrated in a graded series of alcohol. They were next transferred to propylene oxide and finally embedded in Epon. Thin sections were cut with a diamond knife, placed on 200-mesh uncoated grids, and stained with uranyl acetate and lead citrate.

From each of the four sample sites from each rat a series of random electron micrographs was taken by photographing the tissue occurring in the upper right corner of each of 15 consecutive grid spaces from each tissue section. These photographs were enlarged to a final magnification of ×8,000 on 11 × 14 in. photographic paper.

Morphometry. Procedures used in our laboratory for carrying our morphometric studies at both the light and the electron microscopic level have been extensively described (6, 10) and were adapted from those described by Weibel (34). Each ×8,000 electron micrograph was placed under a plastic overlay that contained 112 2-cm lines. The end of each line was assumed to be a point, and the distribution of all points was hand tallied with respect to air, capillaries, and tissue, and the various major components of each of these. The number of times any line intercepted either an alveolar type I cell or an alveolar type II cell on the alveolar epithelial surface, a capillary endothelial surface or a red blood cell was counted. Calculations of volume and surface density and arithmetic mean tissue thickness were made using the basic formula described by Weibel (34). Volume density and surface density measurements were converted to absolute values for the entire lung by multiplying them by the total volume of the alveolar region of the lung (10). Data from six of the eight control animals used in these

morphometric studies have been previously reported (10).

The total number of each major type of cell found in the alveolar region of the lung was determined by using the relationship $N_V = N_A/\bar{D}$ (16). We counted nuclei and assumed that each cell had one nucleus. Thus N_V is the number of cells per unit volume of tissue when N_A is the number of nuclear profiles sectioned per unit area of a random sectioning plane, and $\bar{D}$ is the mean caliper diameter of the nuclei. The mean caliper diameters of lung cell nuclei have been previously determined using serial section three-dimensional reconstructions (5, 10) for normal rats and for rats exposed to 85% O_2 for 7 days. Exposure to 85% O_2 is associated with far greater lung injury than is exposure to 60% O_2. This higher dose of O_2 caused a change in nuclear $\bar{D}$ of 11% or less for each cell type except for alveolar type II cells, which had a 21% increase in nuclear $\bar{D}$ (9). On this basis we assumed that exposure to 60% O_2 would not cause significant changes in $\bar{D}$ for nuclei of most cell types, and we chose to create three-dimensional nuclear models only for alveolar type II cells and endothelial cells from animals exposed to 60% O_2. For alveolar type I cells, interstitial cells, and alveolar macrophages we assumed the nuclear $\bar{D}$ to be that of the control animals (5). N_A was determined for each type of nucleus by using the electron microscope and counting the number of nuclear profiles of each cell type found in 25 consecutive grid spaces from each of the four sites studied per animal.

Physiological studies. We determined the total lung capacity (TLC) of the anesthetized animals by gas dilution and the diffusing capacity for CO (D_{LCO}) with a single-breath method (32). Vital capacity (VC) was estimated as the volume of gas necessary to inflate the lung from residual volume (RV), defined at an airway pressure of 10 cmH_2O less than atmospheric pressure (P_B), to TLC, defined at an airway pressure of 25 cmH_2O above P_B. A volume of gas equivalent to the VC and containing 0.5% neon was then injected into and out of the lungs 10 times from RV, and TLC was calculated by the dilution of the neon as measured by gas chromatography. The diffusing capacity was measured using 0.5% CO and 0.5% neon and a 10-s period of breath holding. The measurements of VC and TLC were done in triplicate. The mean values were calculated, corrected for tracheal air volume, and converted to BTPS.

Determination of pressure-volume (PV) relationships for air-filled lungs. Rat lungs were excised, a 15-gauge Luer stub adapter was tied into the trachea, and the lungs were degassed in a vacuum chamber. After this, the tracheal cannula was connected to a three-way stopcock, one arm of which was connected to a 10-ml syringe and the other to an isovolume water monometer. Air was introduced into the lung in 1-ml increments, allowing 30 s for equilibration before the pressure was recorded. Total lung capacity was defined as the volume of air resulting in a transpulmonary pressure of 30 cmH_2O. On the first inflation curve, pressures higher than 30 cmH_2O were used only if necessary to open collapsed areas. The deflation curve was evaluated between TLC and RV by serially removing 1 ml of air and allowing 10 s for equilibration before recording the pressures. The PV relationships were determined in duplicate for each lung, with the second inflation beginning at RV. The lungs were kept moist by applying saline to the pleural surface periodically during the procedure.

Determination of PV relationships of saline-filled lungs. The saline-filled PV relationships were then determined in these excised lungs. The lungs were again degassed in a vacuum chamber and then connected to a saline-filled syringe and a water monometer using a three-way stopcock. Zero pressure on the monometer was set at the level of the carina, and the lungs were suspended in a saline-filled container. Lungs were inflated with saline to TLC as described above by serially infusing 1 ml of saline, allowing 2 min for equilibration, and then reading the corresponding pressure. The deflation limb between TLC and RV was determined by serially removing 1 ml of saline in increments and recording the pressure after 2 min.

Mechanical ventilation of anesthetized animals. During the process of measuring TLC in intact animals, we observed that some of the exposed animals developed pulmonary edema, whereas this never occurred with control animals. This suggested to us that animals exposed to 60% O_2 for 7 days had damage to the pulmonary vascular bed and were more susceptible to vascular injury by overdistention with large tidal volumes. To evaluate this further we studied a group of animals using mechanical ventilation and large tidal volumes. After pentobarbital anesthesia, a 15-gauge Luer stub adapter was tied into the trachea of each animal, and they were ventilated by a pressure respirator (Ealing animal respirator, model 5255, Harvard Apparatus). The ratio of peak inspiratory pressure to end-expiratory pressure was adjusted to 30/0, 40/0, or 40/10 cmH_2O, and the animals were ventilated for 30 min. We then opened the abdomen of the anesthetized animals and severed the abdominal vessels. After 30–60 s, the lungs were removed, dissected free at the hilum, weighed, and the ratio of wet weight to dry weight was determined by drying the right cardiac lobe of each animal to a constant weight. This required 48 h at a temperature of 50°C.

Statistics. All statistical comparisons were done using unpaired, two-tailed Student's t tests, except for the data in Fig. 2 in which comparisons between treated groups and the control group were done using analysis of variance and Dunnett's multiple comparison test (12).

RESULTS

Evaluation of pulmonary O_2 tolerance. Thirty rats were preexposed to either 21, 60, or 85% O_2 in chambers for one week, after which they were transferred to a pure O_2 atmosphere. Cumulative mortality in 100% O_2 was evaluated at 8-h intervals (Fig. 1). This experiment was then repeated 4 times to give a total of 40 animals in each exposure group. Ninety-five percent of the control animals died between 64 and 80 h of exposure; this group had a median survival time (LT_{50}) of 66 h. Animals preexposed to 85% O_2 were found to be tolerant to 100% O_2 with no mortility observed after 96 h of exposure to pure O_2 ($P < 0.01$). Animals preexposed for 1 wk to 60% O_2 not only did not develop O_2 tolerance but had an

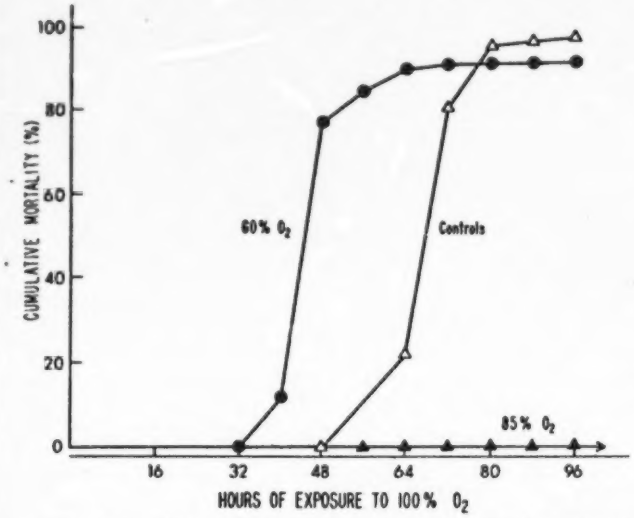


FIG. 1. Cumulative mortality of rats exposed to 100% O_2. △, Control animals kept in air before being exposed to 100% O_2; ▲, animals exposed to 85% O_2 for 7 days before being exposed to 100% O_2; ●, animals exposed to 60% O_2 for 7 days before being exposed to 100% O_2. $n = 40$ for each group. $P < 0.01$ for comparisons of LT_{50} of either treated group of animals to controls.

accelerated death rate upon exposure to 100% O_2. Seventy-eight percent of these animals were dead after 48 h in 100% O_2, by which time none of the control animals had died. The LT_{50} in 100% O_2 of animals preexposed to 60% O_2 was 46 h ($P < 0.01$).

Lung biochemical changes after exposure to hyperoxia. Lung tissues from rats exposed to 85% O_2 had biochemical changes consistent with those previously reported (11, 13). After exposure to 85% O_2, body weight fell (Table 1) by 19%, lung weight increased by 63%, total lung protein increased by 62%, and activity of G-6-PD increased by 174%. It had been previously suggested that changes in antioxidant enzymes such as the superoxide dismutases and changes in enzymes involved in supplying substrates necessary for cell repair such as G-6-PD are critical components of the adaptive process in animals exposed to 85% O_2 (11, 33). As the animals exposed to 60% O_2 did not show evidence of O_2 adaptation, this hypothesis would predict that 60%-O_2 exposure would not be associated with an increase in these enzymes. Animals exposed to 60% O_2 were found to have a slight, but statistically significant, decrease in body weight. Lung weight, total lung protein, total lung superoxide dismutase activity, and total lung G-6-PD activity were not significantly different from control values.

There are two superoxide dismutase enzymes commonly found in mammalian tissues (9, 14). The copper-zinc superoxide dismutase is predominantly found in the cell cytoplasm and has been found to be increased by approximately 50% in the lungs of animals adapted by exposure to 85% O_2 (9, 13). The second superoxide dismutase is a manganese-containing enzyme and is located in mitochondria. It has been found to double in activity in the lungs of rats adapted to O_2. To evaluate the component of the total lung superoxide dismutase activity that was due to the activity of each of these enzymes and to be certain that one of the enzymes had not increased in activity while the other had fallen, we carried out superoxide dismutase assays at pH 7.8 and pH 10.0 and calculated the relative activity of the two enzymes (8) (Table 2). The animals used for these analyses were different animals from those reported in Table 1. No statistically significant change was found in the activity of either form of superoxide dismutase.

Previous work has already shown that exposure of adult rats to 100% O_2 up to the time of death does not cause an increase in activity of the superoxide dismutases in whole-lung extracts (4). To determine whether or not exposure to 60% O_2 could "condition" the lungs so that during a subsequent exposure to 100% O_2 superoxide dismutase activity would increase, we exposed additional groups of animals to air for 8 ½ days, to air for 36 h, and then to 60% O_2 for 7 days, to air for 7 days, and then to 100% O_2 for 36 h or to 60% O_2 for 7 days followed by 100% O_2 for 36 h. At the end of each exposure, lung superoxide dismutase activity was assayed by both the pH 7.8 and the pH 10.0 cytochrome *c* assays (Fig. 2). Thirty-six hours of exposure to 100% O_2 was chosen as the end point for these assays, because after this point deaths begin to occur in animals preexposed to 60% O_2. No statistically significant changes were found in the activity of the superoxide dismutases in any of the groups.

The comparison of the $\dot{Q}O_2$ and of the oxidation of 1-

TABLE 1. *Biochemical changes in rat lungs after exposure to 60 and 85% O_2 for 7 days*

	60% O_2		85% O_2	
	Air Controls	Treated	Air Controls	Treated
n	8	8	16	16
Final body wt, g	395 ± 3	380 ± 4*	397 ± 3	323 ± 5*
Lung wt, g	1.56 ± 0.03	1.53 ± 0.05	1.65 ± 0.06	2.69 ± 0.08*
Total protein/lung, mg	185 ± 3	185 ± 9	199 ± 25	277 ± 17*
SOD/lung, units pH 10.0 assay	3,724 ± 107	3,881 ± 40	4,387 ± 189	7,088 ± 302*
G-6-PD/lung, units	3.4 ± 0.2	3.7 ± 0.2	3.1 ± 0.4	8.5 ± 1.1*

All data are means ± SE. SOD, superoxide dismutase; G-6-PD, glucose-6-phosphate dehydrogenase. * $P < 0.01$ (two-tailed tests).

TABLE 2. *Pulmonary superoxide dismutases in rats after exposure to 60% O_2 for 7 days*

	Air Controls	60% O_2
SOD		
pH 10.0 assay, U/lung	3,898 ± 123	4,708 ± 53
pH 7.8 assay with 10^{-5} M KCN, U/lung	502 ± 6	503 ± 3
Calculated data,* U (pH 7.8)/whole lung		
Copper-zinc SOD	417 ± 15	441 ± 6
Manganese SOD	83 ± 11	62 ± 3

All data are means ± SE; $n = 4$. None of the changes were statistically significant. SOD, superoxide dismutase. * Calculated from simultaneous equations based on copper-zinc enzyme being 9.7 times more active in pH 10.0 assay relative to pH 7.8 assay, with the manganese enzyme having same activity in both assays.

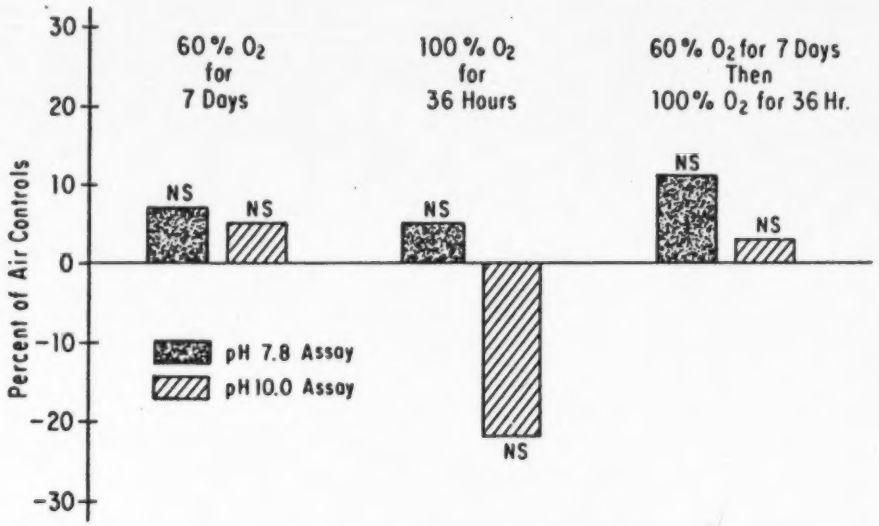


FIG. 2. Changes in superoxide dismutase activity after exposure to 60 and 100% O_2. Percent changes in superoxide dismutase activity per whole lung when compared to control rats kept in air are given for both assay techniques. $n = 5$ for each group; NS, not statistically significant.

[^{14}C] and 6-[^{14}C]glucose] by 1-mm lung slices from control and exposed rats is given in Table 3.

Histological studies of lung injury by 60% O_2. By means of light microscopy perivascular edema was found in the lungs of animals exposed to 60% O_2 for 7 days (Fig. 3, *A* and *B*). This finding was present in all animals evaluated. There was no evidence of alveolar wall or alveolar space edema nor was there abnormal infiltration of cells identifiable by light microscopy. This was true for both the lungs of the 6 animals exposed to 60% O_2 for 7 days and fixed with intratracheal glutaraldehyde and the lungs of 15 animals exposed to 60% O_2 for 7 days and fixed by immersion in Formalin. Morphometric techniques were used to further define the pulmonary injury caused by exposure to 60% O_2 for 7 days. The volume of air space in the alveolar region of the glutaraldehyde fixed lungs was 28% lower in the O_2-exposed animals. There were no significant differences in the total volumes of tissue and capillary blood compartments. Table 4 gives the absolute volumes, surface areas, and mean thickness of tissue compartments in the alveolar region of the lungs. The significant changes after exposure to 60% O_2 for 7 days were an 18% increase in a alveolar epithelial surface area, a 25% decrease in the volume of pulmonary capillary endothelial cells, and a 31% decrease in the arithmetical mean thickness of the endothelial compartment.

The size and shape of nuclei from alveolar type II epithelial cells and capillary endothelial cells were determined by creating three-dimensional models of 10 nuclei from each type of cell. These nuclei were randomly selected from sections from four animals. The $\bar{D}$ for aleveolar type II cells was found to be 6.77 ± 0.24 μm (± SE), which was not statistically different from the control value of 6.92 ± 0.18 (5). For capillary endothelial cells the $\bar{D}$ was 7.22 ± 0.22 μm (± SE), which was 10% less ($P < 0.05$) than the control value of 8.00 ± 0.24 (5). By means of these values for $\bar{D}$ for alveolar type II cells and endothelial cells and with the assumption of normal values for the other cell types (5), the total number and distribution of cells in the alveolar region of the lungs

TABLE 3. *In vitro metabolism of 1-mm rat lung slices*

	n	Air Controls	60% O_2 × 7 Days
$\dot{Q}_{O_2}$, μl/g lung × 60 min^{-1}			
Base-line $\dot{Q}_{O_2}$, 90 min	3	1,323 ± 77	1,473 ± 130
DNP stimulated $\dot{Q}_{O_2}$, 90 min	3	2,017 ± 78	2,150 ± 13*
Average % stimulation		53 ± 8	47 ± 13
Oxidation of 1-[^{14}C]glucose,‡ μmol/g lung × 90 min^{-1}	19	6.82 ± 0.98	7.30 ± 0.78
Oxidation of 6-[^{14}C]glucose,‡ μmol/g lung × 90 min^{-1}	19	1.98 ± 0.47	2.01 ± 0.32

All data are means ± SD. $\dot{Q}_{O_2}$, O_2 consumption; DNP, dinitrophenol. *n* no. of animals. From each animal, we studied 3 flasks for $\dot{Q}_{O_2}$ and 2 flasks for glucose oxidation. * $P < 0.05$ (two-tailed tests). ‡ Calculated on basis that each mole of CO_2 produced represents 1 mol of glucose oxidized.

was determined (Table 5). No change in total cell number or in the distribution of cells was found except for a twofold increase in the number of alveolar macrophages present. Because there was an increase in total alveolar type II cell volume and no change in total number of alveolar type II cells, the average alveolar type II cell had to have increased in volume by 40%. Similarly, because the number of endothelial cells did not change, the fall in volume of pulmonary endothelium can only be explained by a fall in mean endothelial cell size of approximately 25%.

Qualitative evaluation of the lung using electron microscopy demonstrated an increase in the amount of electron-lucent material (which is assumed to be fluid) found in perivascular regions and between endothelial cells and pericytes. Figure 4 illustrates a typical control rat. Figures 5 and 6 illustrate the alveolar region of animals exposed to 60% O_2 for 7 days in which small pockets of electron-lucent material are seen along regions of the capillary wall. Although similar regions of pericapillary fluid could be found in control animals, there was a distinct difference in the size and extent of these areas of fluid accumulation in the treated animals. The distribution of this pericapillary fluid was patchy in the treated animals, as seen in Fig. 5, and the total mass of this fluid was not sufficiently large to increase the morphometric

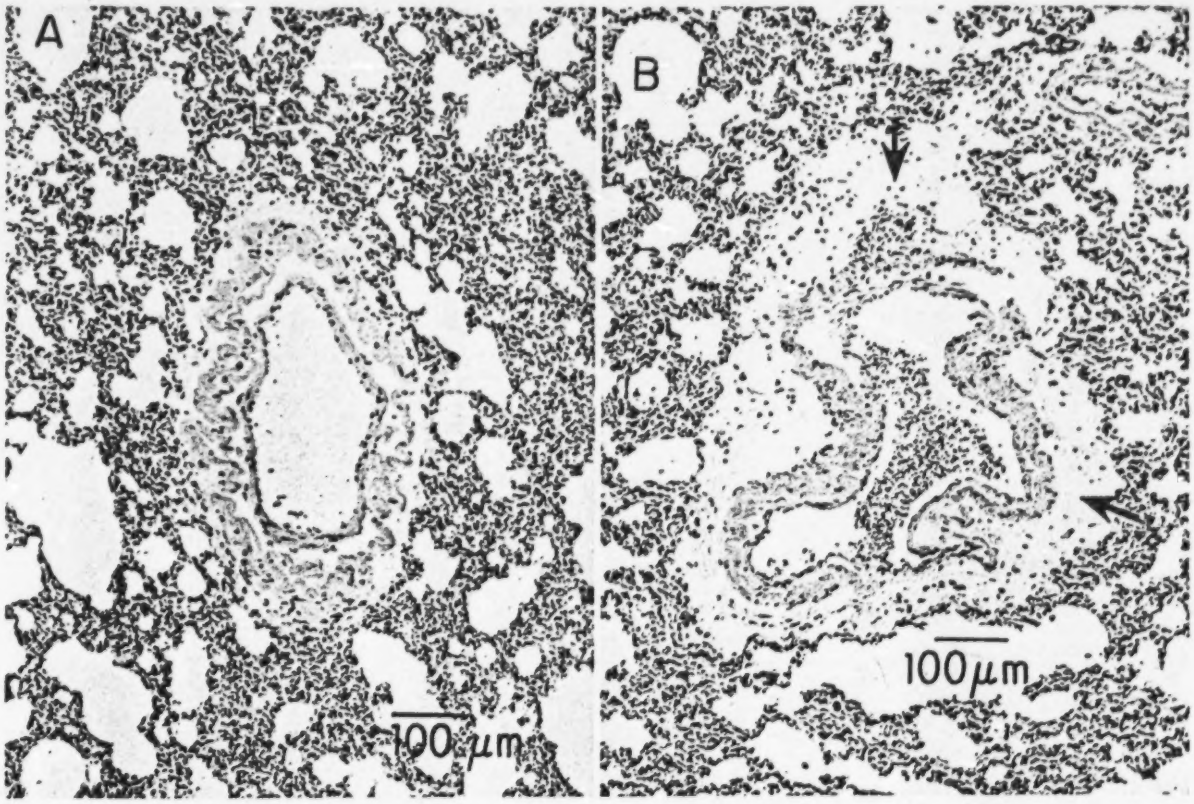


FIG. 3. Light microscopic sections of rat lung. *A*: control animal; *B*: after exposure to 60% O_2 for 7 days. Alveolar walls appear normal in both sections but in *B* there is significant perivascular edema (*arrows*).

TABLE 4. *Absolute volumes, surface areas, and mean thickness of tissue compartments in alveolar region of lung*

	Air Controls	60% O_2 × 7 Days
n	8	6
Body wt, g	363 ± 4	379 ± 4
Total volumes, cm^3/both lungs		
Air	7.216 ± 0.278	5.219 ± 0.086*
Capillary lumen	0.659 ± 0.055	0.732 ± 0.058
Tissue	0.671 ± 0.041	0.663 ± 0.055
Type I epithelium	0.144 ± 0.010	0.131 ± 0.011
Type II epithelium	0.053 ± 0.009	0.074 ± 0.008
Interstitium	0.293 ± 0.029	0.303 ± 0.029
Endothelium	0.156 ± 0.007	0.119 ± 0.010*
Macrophages	0.025 ± 0.006	0.036 ± 0.006
Surface area, cm^2/both lungs		
Alveolar epithelium	4,017 ± 261	4,738 ± 228*
Type I	3,866 ± 253	4,550 ± 226*
Type II	150 ± 23	188 ± 12
Capillary endothelium	4,524 ± 347	4,819 ± 266
Tissue thickness, μm		
Harmonic mean (air to plasma)	0.404 ± 0.017	0.423 ± 0.008
Arithmetic mean (volume/surface)		
Epithelium	0.500 ± 0.033	0.436 ± 0.033
Interstitium	0.693 ± 0.058	0.632 ± 0.043
Endothelium	0.358 ± 0.031	0.246 ± 0.009*

All data are means ± SE. * $P < 0.05$ (two-tailed tests).

TABLE 5. *Morphometrically determined characteristics of major classes of cells in alveolar region of rat lungs*

	Air Controls	60% O_2 Exposure
n	8	6
Total no. cells/both lungs (×10^6)	889 ± 38	917 ± 61
Alveolar type I cells		
Total no., × 10^6	79 ± 8	67 ± 6
% Total lung cells	9	7
% Alveolar surface covered	96	96
Alveolar type II cells		
Total no., × 10^6	126 ± 8	128 ± 9
% Total lung cells	14	14
% Alveolar surface covered	4	4
Endothelial cells		
Total no., × 10^6	410 ± 18	411 ± 17
% Total lung cells	46	45
Interstitial cells		
Total no., × 10^6	247 ± 20	260 ± 28
% Total lung cells	28	28
Macrophages		
Total no., × 10^6	27 ± 3	52 ± 9*
% Total lung cells	3	6

All data are means ± SE. * $P < 0.05$ (two-tailed tests).

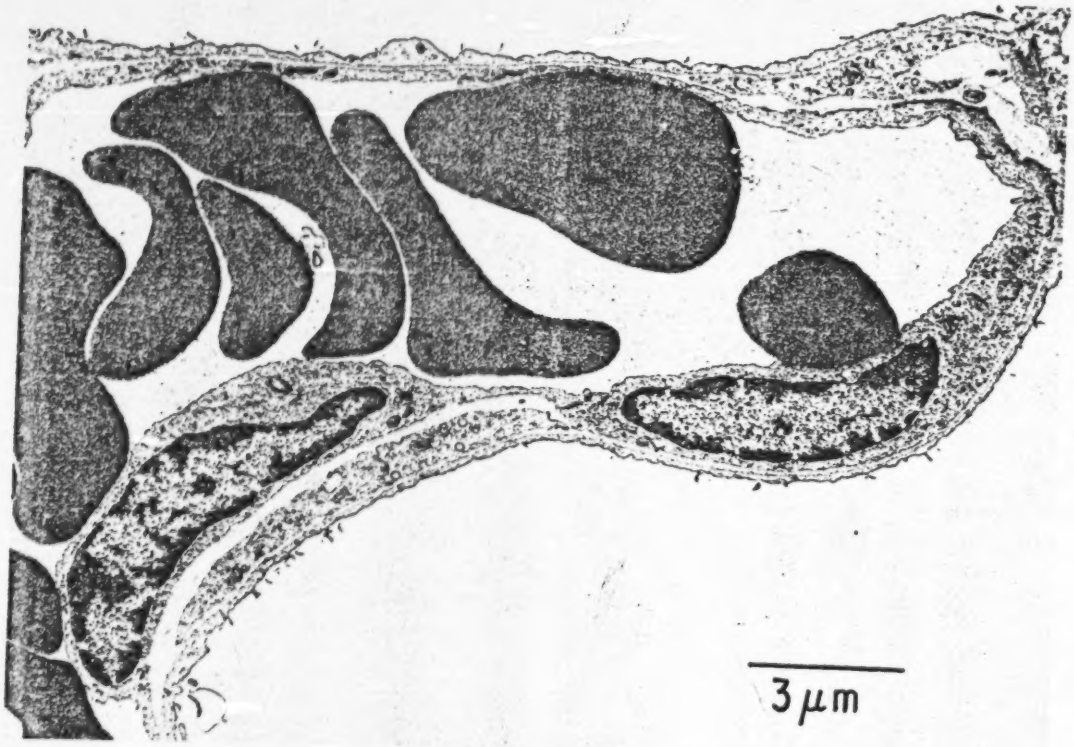


FIG. 4. Electron micrograph of alveolar region normal rat lung.

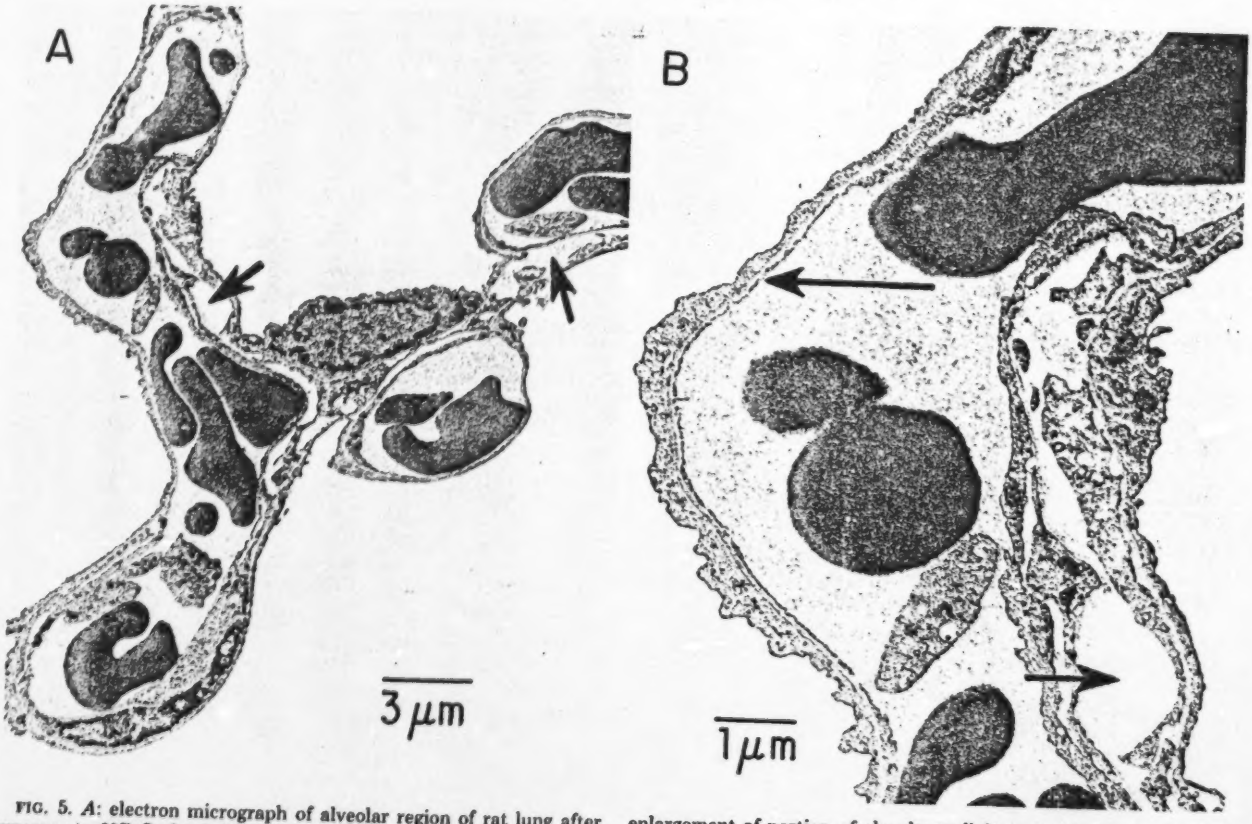


FIG. 5. *A*: electron micrograph of alveolar region of rat lung after exposure to 60% O_2 for 7 days. Note small areas of pericapillary fluid accumulations (*arrows*) along several portions of alveolar wall. *B*: enlargement of portion of alveolar wall from *A* showing area of perivascular fluid (*short arrow*) and marked thinning of portions of endothelium (*long arrow*).

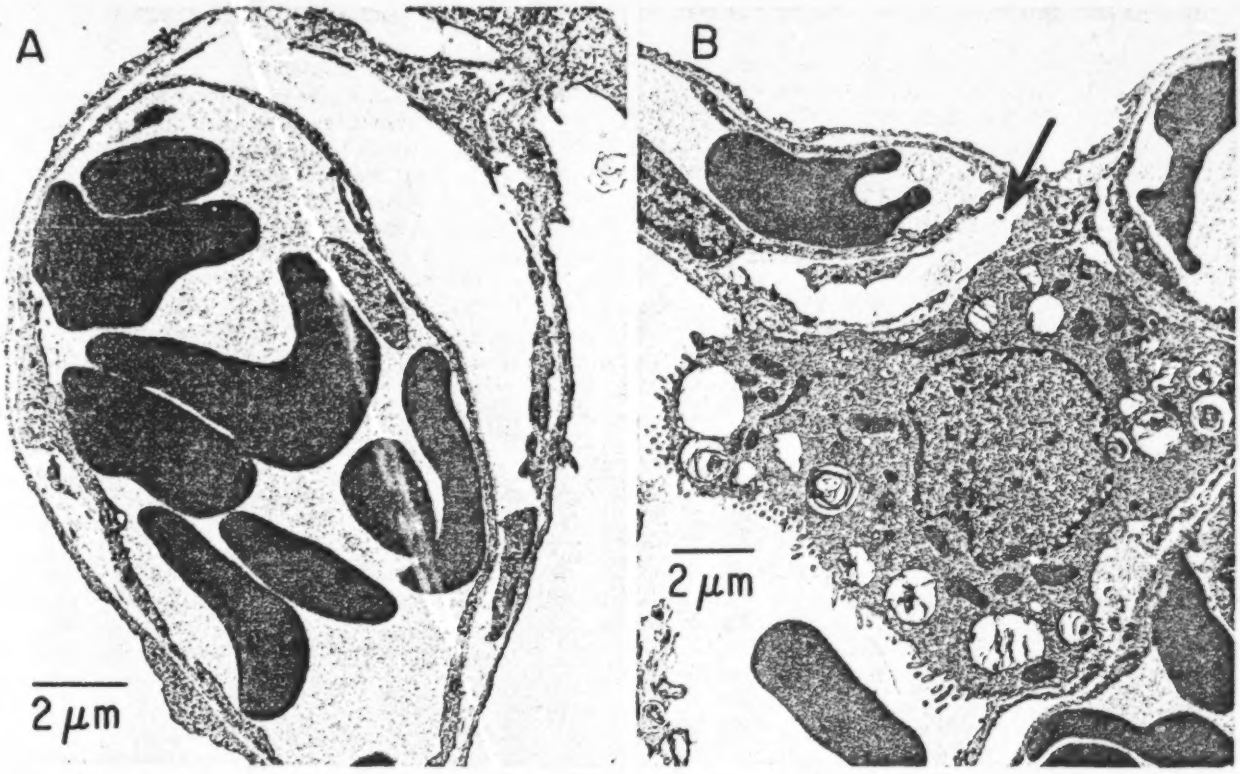


FIG. 6. Electron micrographs of alveolar region of rat lung after exposure to 60% O_2 for 7 days. *A*: abnormal accumulation of pericapillary fluid is illustrated; *B*: typical alveolar type II epithelial cell. Mean type II cell mass was increased after exposure to O_2, but no qualitative changes in ultrastructure were found. *Arrow* shows area of interstitial fluid accumulation.

estimate of the volume of the interstitium by a statistically significant amount.

Physiological studies. We measured TLC by gas dilution techniques in intact animals and found that it was decreased 15% after exposure to 60% O_2 for 7 days (Fig. 7). To determine whether this was due to a change in tissue compliance or to a change in surface tension, we studied the PV relationships of air- and saline-filled lungs from six animals. In Fig. 8, the deflation limb of the PV curve of air- and saline-filled lungs for both an O_2-exposed and a control rat are shown. The animals used for this illustration were representative of both groups. The PV curve for both the air- and saline-filled lungs was shifted to the right for the animals exposed to 60% O_2. The rightward shift was greater with the saline-filled lungs. Table 6 gives the mean values of PV relationships for all animals studied. For air-filled lungs, we compared lung volumes at airway pressures of 3, 10, and 30 cmH_2O using the expiratory limb of the curves. At each of these pressures the mean lung volume was lower in the treated animals, but the difference was not statistically significant. When these lungs were filled with saline, a significant increase in airway pressure was observed at TLC. This change was also present when the lungs were saline-filled to half of the TLC but was not statistically significant. These findings suggest that animals exposed to 60% O_2 for 7 days have a fall in TLC due at least in part to a fall in lung tissue compliance. The D_{LCO} was normalized for each animal and expressed as diffusing capacity per lung volume (Table 7). There was no difference between the groups, implying that whereas exposure to 60% O_2 resulted in a fall in TLC, the diffusing capacity was not changed.

Pulmonary edema produced in O_2-exposed rats by mechanical ventilation. Animals exposed to 60% O_2 had normal lung weights and a normal lung wet weight-dry weight ratio if not subjected to mechanical ventilation or if ventilated at pressures of 30/0 cmH_2O (Table 8). Control animals ventilated at 40/0 cmH_2O had a 50% greater lung wet weight, whereas animals exposed to 60% O_2 for 7 days and then ventilated at 40/0 cmH_2O had a 200% greater lung wet weight. If positive end-expiratory pressure is added and the animals are ventilated at pressures of 40/10 cmH_2O, the differences in lung wet weight did not occur.

DISCUSSION

A number of studies of tolerance to moderately increased partial pressures of oxygen have been performed in small animals (3). Most of them have failed to find significant evidence of lung injury when the inspired oxygen concentration was kept below 60%. The common indices of pulmonary oxygen toxicity used include obvious symptoms, mortality, and lung pathology. The mortality of animals exposed to 60% oxygen atmospheres or less has not been found to differ from that of control animals breathing air except for a study of young lambs by Shermeta et al. (29). These lambs were exposed to 60–65% O_2, and 50% of them died by the 15th day of exposure.

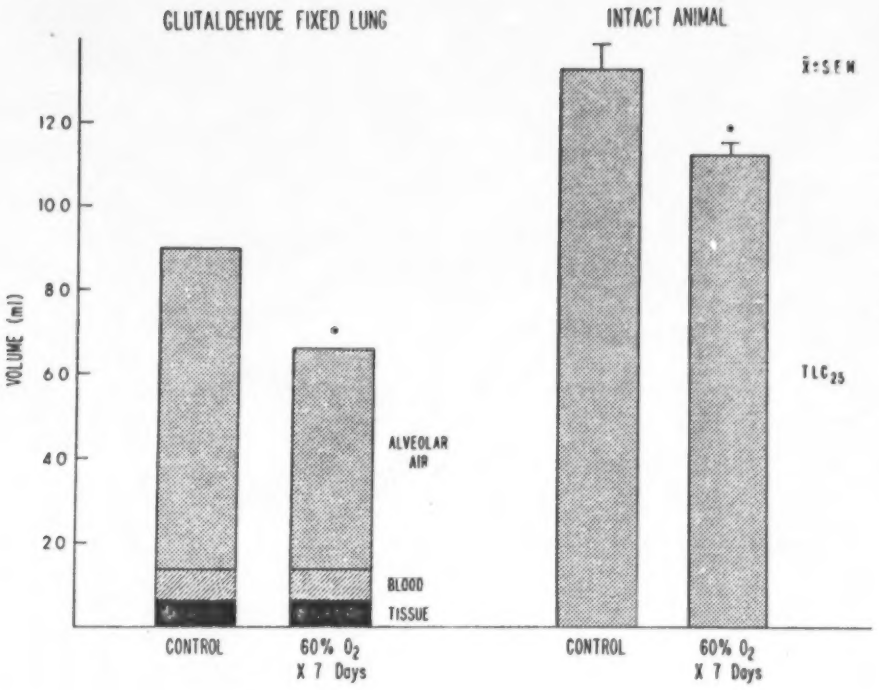


FIG. 7. Comparison of lung volumes in glutaraldehyde-fixed lungs with total lung capacity (TLC) measured by gas dilation in intact animals. $n = 8$ for all groups except for glutaraldehyde-fixed lungs from O_2-exposed animals where $n = 6$. * Statistically significant changes from respective controls.

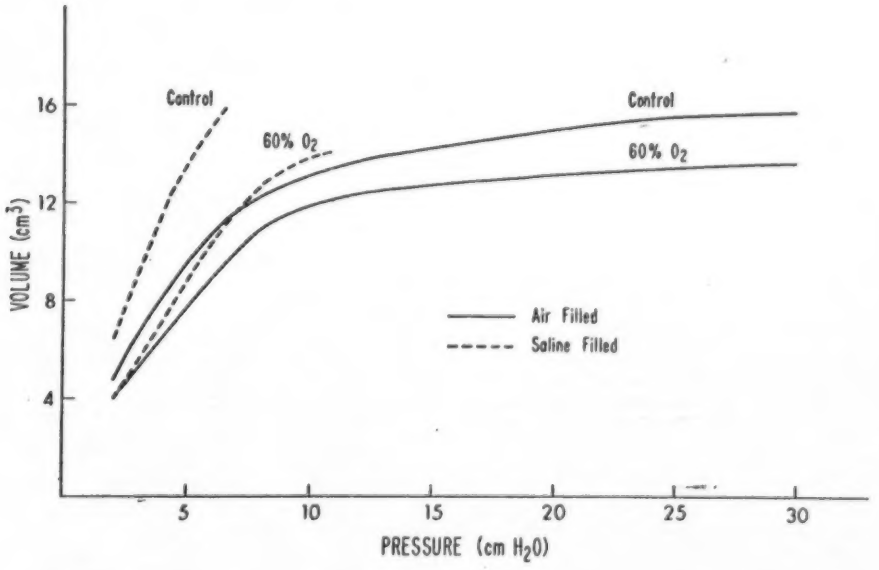


FIG. 8. Deflation limb of pressure-volume curve for air- and saline-filled lungs from control animal and animal exposed to 60% O_2 for 7 days.

TABLE 6. *Pressure and volume relationships of air- and saline-filled lungs*

	Air-Filled			Saline-Filled	
	V_{P3}	V_{P10}	V_{P30}	$P_{TLC/2}$	P_{TLC}
Air controls	6.89±1.32	12.36±1.49	15.30±0.66	2.81±0.61	8.00±0.98
60% O_2 × 7 days	5.37±1.70	11.03±1.70	14.53±0.58	3.00±0.72	10.75±0.82*

All data are means ± SE; $n = 6$. V_{P3}, V_{P10}, and V_{P30}, lung volume in ml at airway pressures of 3, 10, and 30 cmH_2O, respectively; $P_{TLC/2}$ and P_{TLC}, airway pressure in cmH_2O at midlung and total lung capacity, respectively. *$P < 0.05$.

The ratio of lung wet weight to dry weight was elevated in these animals, and the cause of death was thought to be pulmonary edema. In additon to being exposed to 60% O_2 for 15 days, these animals also had had multiple thoracotomies, which may have contributed to their pulmonary injury and to their death. Barach (1) studied rabbits exposed to 60% O_2 for 2 mo but found no abnormality in lung histology. Brooksby et al. (2) evaluated rats exposed to 60% O_2 for 64 days and found no changes detectable by light microscopy. Hackney et al. (17), using autoradiography and light microscopy, found hyperemia and slight edema in the lungs of both exposed and control

TABLE 7. *Diffusing capacity and total lung capacity after exposures to 60% O_2*

	Air Controls	60% O_2 Exposure
Body wt, g	347.2 ± 20.4	356.5 ± 28.7
Lung wt, g	1.49 ± 0.19	1.49 ± 0.17
TLC, ml	14.43 ± 1.99	12.60 ± 2.04*
Diffusing capacity, ml·min^{-1}·Torr^{-1}	0.2060 ± 0.0437	0.1805 ± 0.0353
$D_{L_{CO}}$/TLC	0.0147 ± 0.0043	0.0148 ± 0.0042

All data are means ± SD; n = 38. TLC, total lung capacity. $D_{L_{CO}}$, single-breath diffusing capacity for CO. * $P < 0.01$ (two-tailed tests).

squirrel monkeys after 8 days of exposure to 60% O_2. These finding were considered to be inconsequential.

Some quantitative descriptions of alterations in pulmonary structure after prolonged exposure to mild hyperoxia have been done using morphometric techniques. Prolonged exposure of rats to 0.33 atm of O_2 was found to lead to a 54% increment in arithmetical mean thickness in the alveolar capillary tissue barrier, this was due to interstitial edema and resolved almost completely after a recovery period of 30–40 days (18, 20).

Our current study was conceived to answer several questions which include, Does exposure to 60% O_2 cause significant lung injury? Can this lung injury be aggravated by adding a second insult? Will this dose of O_2 stimulate the pathways leading to oxygen adaptation? Morphometric techniques for quantifying changes in lung structure offer a tool for defining subtle but significant changes that occur. The cell population pattern in the alveolar region was found to be normal, but significant changes were identified in the pulmonary capillary bed. The mean caliper diameter of endothelial nuclei was down 10%, which translates to approximately a 25% decrease in mean nuclear volume. Total capillary endothelial cell volume was down by 24%, and the arithmetic mean endothelial cell thickness was decreased by 31%. These changes involving the pulmonary capillary bed are consistent with previous studies that have suggested that the initial site of injury to the lung in a pure O_2 atmosphere is to the pulmonary capillary endothelium and that destruction of the pulmonary capillary endothelium is the primary cause of interstitial edema, alveolar edema, and the death of the animal (4). Small areas of focal pericapillary edema could be identified using electron microscopy (Figs. 5 and 6), and light microscopy demonstrated distinct areas of perivascular edema involving larger venules (Fig. 3). This suggests that an increased permeability of the capillary endothelium caused by the 60% O_2 exposure is leading to small alveolar interstitial fluid collections and that it is contributing to or causing an increased fluid flow in the dilated perivascular lymphatics seen downstream.

A fall in compliance in our animals exposed to 60% O_2 is suggested by the decrease found in the alveolar air volume in glutaraldehyde-fixed lungs (Table 5). Lung compliance in these animals was studied by evaluating their pressure-volume characteristics using both air and saline. A major component of the increased rigidity of these lungs was due to a fall in lung tissue compliance, as indicated by the significantly increased pressure required to take the saline-filled lungs to total lung capacity (Table 7).

A fall in pulmonary compliance has been reported previously for O_2 exposed animals (24, 30). Redding et al. (26) found a decrease in compliance in rats exposed to 70% O_2 for 7–10 days that was attributed to pulmonary edema. Gacod and Massaro (15) suggested that a decrease in synthesis of surfactant may contribute to the decrease in pulmonary compliance in animals exposed to pure O_2.

The sensitivity of rats to O_2 is age dependent. Young rats are less sensitive and will not die during an acute exposure to 100% O_2, whereas adult rats uniformly die after 60–72 h of exposure (13). Exposure of adult rats to sublethal but toxic doses of O_2, such as 85% O_2, will cause extensive lung injury but will also lead to O_2 adaptation. The activity of enzymes such as the superoxide dismutases, catalase, glutathione peroxidase, and glucose-6-phosphate dehydrogenase have been shown to increase in the lungs of adult rats adapted to hyperoxia (7, 9, 33). The lower dose of O_2, 60% O_2, used in the present study was not associated with an increase in activity of the superoxide dismutases or of glucose-6-phosphate dehydrogenase in the lungs of rats. We were unable to detect changes in the rate of oxidative metabolism, as indicated by $\dot{Q}O_2$, or the oxidation of 1-[^{14}C] or 6-[^{14}C]glucose to CO_2. The extent of uncoupling of mitochondrial oxidative phosphorylation was also not changed following exposure to 60% O_2, indicating that overall mitochondrial activity had not been damaged. It is significant that these lungs did not show the extensive lung injury that has previously been found after exposures to 85% O_2 (4, 10). After exposure to 60% O_2 there was no evidence of alveolar edema, and lung weight and total lung protein were within normal limits. These findings suggest that more extensive lung injury is necessary to stimulate an increase

TABLE 8. *Effects of positive-pressure ventilation on rat lungs exposed to 60% O_2 for 7 days*

Treatment	n	Body Wt, g	Lung Wt, g	Lung Wt, wet/dry
No mechanical ventilation				
Air controls	15	404 ± 4	1.56 ± 0.05	4.34 ± 0.04
60% O_2	15	395 ± 7	1.54 ± 0.03	4.45 ± 0.05
Ventilated at pressure of 30/0*				
Air controls	5	426 ± 9	1.73 ± 0.08	4.62 ± 0.04
60% O_2	5	432 ± 12	1.79 ± 0.05	4.64 ± 0.04
Ventilated at pressure of 40/0*				
Air controls	10	389 ± 9	2.34 ± 0.22†	5.27 ± 0.32†
60% O_2	10	397 ± 7	4.59 ± 0.37‡	7.73 ± 0.40‡
Ventilated at pressure of 40/10				
Air controls	5	406 ± 6	1.70 ± 0.04	4.84 ± 0.13
60% O_2	5	429 ± 3	1.90 ± 0.09	4.77 ± 0.08

All data are means ± SE. * Peak inspiratory pressure (cmH_2O)/end-expiratory pressure (cmH_2O). † $P < 0.05$ for comparisons with nonventilated controls. ‡ $P < 0.05$ for comparisons with ventilated controls.

in the activity of antioxidant enzymes or enzymes involved in supplying substrates for cell repair, such as glucose-6-phosphate dehydrogenase. The failure of 60% O_2 exposure to increase the activity of these enzymes is consistent with the hypothesis that these enzymes are critical components of the process of O_2 adaptation.

Although exposure to 60% O_2 was not associated with extensive lung injury as identified by histological or biochemical parameters, a significant toxic effect was identified. The animals not only failed to undergo O_2 adaptation but instead became more sensitive to the second insult and died earlier than control animals when exposed to pure O_2. Our present studies suggest that injury to the pulmonary capillary bed is occurring during exposures to 60% O_2. The subtle changes occuring in the endothelial compartment may explain the enhanced susceptibility of these animals to the lethal effects of a subsequent exposure to 100% O_2.

We postulated that if the injury to the endothelium caused by 60% O_2 was physiologically significant it might be able to be aggravated further by overexpanding the lungs with large tidal volume breaths. Exposure of rats to positive pressure ventilation at 40/0 cmH_2O for 30 min led to interstitial and alveolar edema even in control animals. However, the animals exposed to 60% O_2 were markedly more sensitive and had a fourfold greater increase in lung wet weight and lung wet-dry weight ratios. Repeated stretching of an already injured pulmonary capillary bed that is not able to withstand the trauma involved in large tidal volume and/or pressure swings is a likely explanation for the greater amount of pulmonary edema in the animals exposed to 60% O_2.

Although rats exposed to 60% O_2 do not develop dramatic changes in lung biochemical parameters or in lung histology, there is a significant injury to the pulmonary capillary bed that leaves the animals more susceptible to subsequent injury by factors such as large ventilatory or pressure excursions or exposure to more toxic O_2 atmospheres. The process of O_2 adaptation is not stimulated by the exposure to 60% O_2, and lung antioxidant enzymes thought to be involved in O_2 adaptation are not changed in activity. These findings have relevance as the clinical use of modest levels of hyperoxia is common. Patients requiring supplemental oxygenation frequently require progressively higher doses of O_2 and mechanical assistance using high volumes or pressures to maintain adequate ventilation.

The authors thank Karen Sjostrom, Laurie Luxner, Dawn Woody, and Robert Mercer for valuable technical assistance provided in carrying out this project, and Dr. Michael Ehrie for helping with the light microscopy.

This work was supported in part by National Heart, Lung, and Blood Institute Grants HL-23805 and HL-25044.

Address for reprint requests: J. D. Crapo, Dept. of Medicine, Duke University Medical Center, Durham, NC 27710.

Received 20 February 1980; accepted in final form 5 June 1981

REFERENCES

1. BARACH, A. L. The effects of atmospheres rich in oxygen on normal rabbits and on rabbits with pulmonary tuberculosis. *Am. Rev. Tuberc.* 13: 293–316, 1926.
2. BROOKSBY, G. A., R. L. DENNIS, AND R. W. STALEY. Effect of prolonged exposures of rats to increased oxygen pressure. In: *Proceedings of the Third International Conference on Hyperbaric Medicine*, edited by I. W. Brown, Jr., and B. G. Cox. Washington, DC: 1966, p. 208–216. (NAS/NRC Publ. 1404.)
3. CLARK, J. M., AND C. J. LAMBERTSEN. Pulmonary oxygen toxicity: a review. *Pharmacol. Rev.* 23: 37–133, 1971.
4. CRAPO, J. D., B. BARRY, H. A. FOSCUE, AND J. SHELBURNE. Structural and biochemical changes in rat lungs occurring during exposure to lethal and adaptive doses of oxygen. *Am. Rev. Respir. Dis.* 122: 123–143, 1980.
5. CRAPO, J. D., AND D. GREELEY. Estimation of the mean caliper diameter of cell nuclei. II. Various cell types in rat lung. *J. Microsc.* 114: 41–47, 1978.
6. CRAPO, J. D., J. MARSH-SALIN, P. INGRAM, AND C. PRATT. Tolerance and cross-tolerance using NO_2 and O_2. II. Pulmonary morphology and morphometry. *J. Appl. Physiol.: Respirat. Environ. Exercise Physiol.* 44: 370–379, 1978.
7. CRAPO, J. D., AND J. M. MCCORD. Oxygen-induced changes in pulmonary superoxide dismutase assayed by antibody titrations. *Am. J. Physiol.* 231: 1196–1203, 1976.
8. CRAPO, J. D., J. M. MCCORD, AND I. FRIDOVICH. Superoxide dismutases, preparation and assay. In: *Methods in Enzymology, Biomembrane Series, Part C.* New York: Academic, 1977, p. 382–393.
9. CRAPO, J. D., M. PETERS-GOLDEN, J. MARSCH-SALIN, AND J. S. SHELBURNE. Pathologic changes in the lungs of oxygen-adapted rats. A morphometric analysis. *Lab. Invest.* 39: 640–653, 1978.
10. CRAPO, J. D., K. SJOSTROM, AND R. T. DREW. Tolerance and cross-tolerance using NO_2 and O_2. I. Toxicology and biochemistry. *J. Appl. Physiol.: Respirat. Environ. Exercise Physiol.* 44: 364–369, 1978.
11. CRAPO, J. D., AND D. F. TIERNEY. Superoxide dismutase and pulmonary oxygen toxicity. *Am. J. Physiol.* 226: 1404–1407, 1974.
12. DUNNETT, C. W. New tables for multiple comparisons with a control. *Biometrics* 20: 482–491, 1974.
13. FRANK, L., J. R. BUCHER, AND R. J. ROBERTS. Oxygen toxicity in neonatal and adult animals of various species. *J. Appl. Physiol.: Respirat. Environ. Exercise Physiol.* 45: 699–704, 1978.
14. FRIDOVICH, I. Superoxide dismutase. *Adv. Immunol.* 41: 35–97, 1974.
15. GACOD, G., AND D. MASSARO. Hyperoxia: influence on lung mechanics and protein synthesis. *J. Clin. Invest.* 52: 559–565, 1973.
16. GREELEY, D., J. D. CRAPO, AND R. VOLLMER. Estimation of the mean caliper diameter of cell nuclei. I. Serial section reconstruction method and endothelial nuclei from human lung. *J. Microsc. Oxford* 114: 31–39, 1978.
17. HACKNEY, J. D., M. J. EVANS, AND B. R. CHRISTIE. Effects of 60 and 80% oxygen on cell division in lung alveoli of squirrel monkeys. *Aviat. Space Environ. Med.* 46: 791–794, 1975.
18. KISTLER, G. S., E. R. WEIBEL, AND P. R. B. CALDWELL. Electron microscopic and morphometric study of rat lungs exposed to 97% oxygen at 258 Torr (27,000 ft). In: *Proceedings of the Second Annual Conference on Atmospheric Contaminations in Confined Spaces.* Wright-Patterson Air Force Base, OH: Aerospace Medical Research Laboratories, 1966, p. 147–161. (Rep. AMRL-TR 66-120).
19. LAVOISIER, A. L. Memoire sur les alterations qui arruvent a L'air dans plusieurs circonstances ou se trouvent les hommes reunis en societe. *Histoire Soc. R. Med.* 5: 569–582, 1785.
20. LEWEREN, Z. M., G. SCHURINGER, E. R. WEIBEL, AND H. P. KAPLAN. *Electron Microscopic and Morphometric Evaluation of Lungs from Animals Exposed Continuously for Eight Months to 5 PSIA, 100% Oxygen.* Wright-Patterson Air Force Base, OH: Aerospace Medical Research Laboratories 1967, p. 89–104. (Rep. AMRL-TR 67-200).
21. LOHR, G. W., AND H. D. WALLER. Glucose-6-phosphate dehydrogenase. In: *Methods of Enzymatic Analysis*, edited by J. U. Bergmeyer. New York: Academic, 1967, p. 744.
22. LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR, AND R. J. RANDALL. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193: 265–275, 1951
23. O'NEIL, J. J., R. L. SANFORD, S. WASSERMAN, AND D. F. TIERNEY. Metabolism in rat lung tissue slices: technical factors. *J. Appl. Physiol.: Respirat. Environ. Exercise Physiol.* 43: 902–906, 1977.

24. PAUTLER, S., I. M. CUNONS, D. CAUNA, R. TOTTEN, AND P. SAFAR. Pulmonary oxygen toxicity at one ata. *Acta Anesthesiol. Scand. Suppl.* 24: 51–56, 1966.
25. PRIESTLY, J. The discovery of oxygen, part I. In: *Alembic Club Reprints*. Edinburgh: Clay, 1894, p. 1–55.
26. REDDING, R. A., T. ARAI, W. H. J. DOUGLAS, H. TSURUTANI, AND J. OVERS. Early changes in lungs of rats exposed to 70% O_2. *J. Appl. Physiol.* 38: 136–142, 75.
27. SALIN, M. L., E. D. DAY, AND J. D. CRAPO. Isolation and characterization of a manganese containing superoxide dismutase from rat liver. *Arch. Biochem. Biophys.* 187: 223–228, 1978.
28. SCHERLE, W. A simple method for volumetry of organs in quantitative stereology. *Mikroskopie* 26: 57–60, 1970.
29. SHERMETA, D. W., J. J. WHITE, R. DELEMOS, AND J. A. HALLER, JR. Pulmonary effects of lethal and sublethal oxygen exposure in lambs. *Am. Surg.* 36: 721–723, 1970.
30. SMITH, C. W., P. H. LEHAN, AND J. H. MONKS. Cardiopulmonary manifestations with high O_2 tension at atmospheric pressure. *J. Appl. Physiol.* 18: 849–853, 1963.
31. SMITH, J. L. The pathological effects due to increase of oxygen tension in the air breathed. *J. Physiol London* 24: 19–35, 1899.
32. TAKEZAWA, J., F. J. MILLER, AND J. J. O'NEIL. Single-breath diffusing capacity and lung volumes in small laboratory mammals. *J. Appl. Physiol.: Respirat. Environ. Exercise Physiol.* 48: 1052–1059, 1980.
33. TIERNEY, D., L. AYERS, S. HERZAG, AND J. YANG. Pentose pathway and production of reduced nicotinamide adenine dinucleotide phosphate. *Am. Rev. Respir. Dis.* 108: 1348–1351, 1973.
34. WEIBEL, E. R. Morphometric estimation of pulmonary diffusion capacity. I. Model and method. *Respir. Physiol.* 11: 54–75, 1970.